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Expression pattern of sensory neuron membrane protein (SNMP) in adults of *Helicoverpa armigera* (Lepidoptera: Noctuidae) and its relation with Gqα

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Abstract: Insect olfaction is important for insects to detect the food, reproductive partners, oviposition sites, hosts, prey or predators. Sensory neuron membrane protein (SNMP) and Gq alpha subunit (Gqα) were thought to be involved in signal transduction in insect olfaction. In order to make clear the spatial expression pattern of *snmp* and understand whether Gqα is the direct downstream binding protein of SNMP, semi-quantitative RT-PCR method was used to learn the expression pattern of *snmp* in antennae, head, thorax, abdomen, legs, wings, proboscis, labial palpus and maxillary palpus of adult *Helicoverpa armigera*, and yeast two-hybrid technique was used to investigate the relationship between SNMP and Gqα. Semi-quantitative RT-PCR results showed that *snmp* was expressed not only in the antennae of adult *H. armigera*, but also in proboscis, labial palpus, maxillary palpus and legs. Yeast two-hybrid screening results showed that Gqα did not directly interact with SNMP. These results suggest that SNMP plays some roles not only in odor detection but also in taste recognition, and SNMP maybe forms a complex with an olfactory receptor (OR) and then binds with Gqα, which needs our further investigation.

Key words: *Helicoverpa armigera*; insect olfaction; signal transduction; SNMP; Gqα; yeast two-hybrid technique; spatial expression

1 INTRODUCTION

Insect olfaction plays a key role in its life cycle, which can help insects with the detection of food, reproductive partners, oviposition sites, hosts, prey and predators. Insect olfactory recognition process is very complicated, in which many proteins are involved, including odorant binding proteins, olfactory receptors and odor degrading enzymes (Vogt and Riddiford, 1981; Clyne *et al.*, 1999; Rogers *et al.*, 1999; Hill *et al.*, 2002; Su *et al.*, 2006; Benton *et al.*, 2007). These proteins comprise a biochemical network designed to process environmental signals.

G protein, composed of three subunits - α, β and γ-subunit, is regarded as the molecular switch of signal transduction passageway (Cabrera-Vera *et al.*, 2003). Gqα family members have diverse functions due to their link with different receptors and downstream binding partners including effectors, regulatory proteins and scaffolding/adaptor proteins (Hubbard and Hepler, 2006). In *Bombyx mori*, Gqα mediated the signal transduction pathway of olfaction (Miura *et al.*, 2005).

Sensory neuron membrane protein (SNMP) is homologous with CD36 family. It was first identified in *Antheraea polyphemus*, then in *B. mori*, *Heliothis virescens*, and *Manduca sexta* (Rogers *et al.*, 1997, 2001a, 2001b). In the above researches, it was found that SNMPs were antennal-specific proteins located in the receptive membranes of the dendrites. And they occurred simultaneously with the onset of olfactory function. Hence, SNMPs were thought to be involved in odor detection. Benton *et al.* (2007) and Jin *et al.* (2008) found that SNMP in *Drosophila melanogaster* was essential for the responses of OR67d to (*Z*)-11-octadecenyl acetate (*cis*-vaccenyl acetate, cVA), a kind of a volatile male-specific fatty-acid derived pheromone, but not for the sensitivity to the general odorant. In addition, SNMP was also found expressed in the body of *D. melanogaster* (Benton *et al.*, 2007).

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In this study, semi-quantitative RT-PCR was used to learn *snmp* expression pattern in *H. armigera*, and yeast two hybrid method was adopted to explore whether Gqα was the downstream binding protein for SNMP in attempt to clarify the signalling passway of olfactory/taste recognition.

2 MATERIALS AND METHODS

2.1 Insects and tissues

H. armigera was reared in our laboratory on an artificial diet at 28℃, 60% relative humidity, and a 16 h∶8 h light/dark photoperiod. Antennae, heads (excluding the antennae), thorax, abdomen, legs, wings were dissected from *H. armigera* adult within 24 h after emergence. Dissection of labial palpus, maxillary palpus and proboscis was conducted with the help of the microscope. Dissected tissues were immediately frozen in the liquid nitrogen and stored at −70℃ until use.

2.2 RNA isolation and cDNA synthesis

Total RNA was isolated from 10 mg of tissues, such as antennae, labial palpus, maxillary palpus and proboscis, and from 50 − 100 mg of tissues, either heads (excluding the antennae), thorax, abdomen, legs or wings of *H. armigera* with Trizole (Invitrogen, USA), following its operation instructions. The first-strand cDNA was synthesized with oligo (dT) primers and Reverse Transcription System A3500 (Promega, USA).

2.3 RT-PCR and cloning

The gene encoding SNMP was amplified from the first-strand cDNA, which was prepared from the adult antennae just as described above using TaKaRa ExTaq DNA polymerase (TaKaRa Dalian Co., Ltd.) and the specific primers (snmp-1S: 5′-CCCAACCTTCTTGAAACGC-3′; snmp-1A: 5′-GCAACCACAGACCACCATG-3′). The specific primers were designed according to *H. armigera snmp* sequence (GenBank accession no.: AF462067). The amplification was performed according to the following schedule: 3 min at 94℃ followed by 35 cycles at 94℃ (1 min), 53℃ (1 min), 72℃ (1.5 min); finally 10 min at 72℃. The PCR product of the appropriate size was gel-purified with the DNA Gel Extraction Kit (Omega USA), then cloned into T-easy vector (Promega USA), and transformed into *Escherichia coli* DH5α for sequencing in the gene-sequencing company (Bioasia Company).

To investigate the spatial expression of *snmp* by semi-quantitative RT-PCR, a pair of specific primers for *snmp* designed according to *H. armigera snmp* sequence, were used to amplify cDNAs from all sampled tissues: Sense-*snmp*: 5′-GATCAAGACCAACCGTTAC-3′; Anti-*snmp*: 5′-AAGGAGACCATCCACCAG-3′ (the size of corresponding PCR product was 468 bp). The 18S rRNA was chosen to be the reference gene. The specific primers for 18S rRNA cDNA were: 18SS: 5′-TTAGTGAGGTCTTCGGACCG-3′; 18SR: 5′-CTTCCGCAGGTTCCCCTACG-3′.

2.4 Construction of a DNA-BD fusion protein

Because pGBKT7 vector has the restriction sites for *Eco*R Ⅰ and *Pst* Ⅰ, we generated the gene fragment (Gqα) by PCR using primers (Y-GqS/GqA) which contained the desired restriction sites.

Y-GqS: 5′-*TCGGAATTC*ATGGAGTGCTGCATGTCGGAGGA-3′ *Eco*R Ⅰ

Y-GqA: 5′-*GTCCTGCAG*TTAGACGAGGTTGTACTCCTTAAGGT-3′ *Pst* Ⅰ

Gqα was amplified with the specific primers (Y-GqS/GqA) and the pyrobest DNA polymerase (TaKaRa) according to the procedure described above. DNA-BD vector (pGBKT7) and the PCR product of Gqα were digested with *Eco*RⅠ and *Pst*Ⅰ, respectively. The desired fragments were recovered by electrophoresis and further purified with the DNA Purification Kit (Omega), and then pGBKT7 vector and Gqα insert were ligated. The ligation mixture was transformed into *E. coli*. The insert-containing plasmid (Gqα-pGBKT7) was identified by restriction analysis and PCR. The *E. coli* of the positive plasmid was sequenced to make sure the open reading frame was right.

2.5 Transcriptional activation test and toxicity test of the DNA-BD fusion

AH109 was transformed with the hybrid construct (Gqα-pGBKT7) using a small-scale transformation protocol according to the BD Matchmaker™ Library Construction & Screening Kits User Manual.

In order to test the DNA-BD fusion for toxicity, the growth rate in liquid culture of AH109 cells transformed with the "empty" DNA-BD vector was compared with that of AH109 cells transformed with the DNA-BD/bait plasmid.

2.6 Amplification and purification of *snmp* gene

RNA from the adult antennae was isolated with Trizole according to the protocol described above. First-strand cDNA was synthesized using an Oligo (dT) primer, and dscDNA was amplified by PCR and purified using DNA Gel Extraction Kit (Omega USA) according to the Kit user manual.

2.7 Construction and screening of a two-hybrid library

pGADT7-Rec was used to construct a two-hybrid library. The two-hybrid library was screened by cotransformation. This procedure is quick and efficient. We prepared the competent yeast cells with the LiAc method. After the preparation, AH109 was cotransformed with *snmp*-dscDNA, pGADT7-Rec and Gqα-pGBKT7. The cotransformation mixture was spread on TDO (SD/-His/-Leu/-Trp) and QDO (SD/-Ade/-His/-Leu/-Trp) plates (150 μL cells/150 mm plate). At the same time, the yeast strain AH109 was also cotransformed with SV40 Large TPCR fragment, pGADT7-Rec and pGBKT7-53, which was the positive control. The negative control was the yeast strain AH109 cotransformed with SV40 Large TPCR Fragment, pGADT7-Rec and pGBKT7-Lam.

2.8 Analysis of the positive interaction

The positive colonies on SD dropout plates were restreaked 2–3 times to segregate the AD/library plasmids, then the well-isolated colonies were transferred to SD/-Ade/-His/-Leu/-Trp plates containing X-α-Gal to verify that they maintained the correct phenotype and to test the expression of *MEL1*. To identify the gene (and thus protein) responsible for a positive two-hybrid interaction, the gene was rescued by plasmid isolation. The plasmids isolated from yeast were transformed into *E. coli* and selected on LB/Amp. The AD plasmid insert was sequenced directly and the sequence was compared with that of other proteins in GenBank.

3 RESULTS

3.1 Gene cloning and spatial expression pattern of *snmp* in *H. armigera* adult

Using cDNA template and the primers (*snmp*-1S and *snmp*-1A), we successfully obtained a PCR fragment (about 1 700 bp). By ligation with T-easy vector, and then transformation into *E. coli* DH5α, and sequencing of the positive clone, the result showed that the sequence was 1 690 bp, including a 1 572 bp open reading frame which encoded 523 aa. The deduced amino acid sequence was analyzed with Blast in the NCBI. The result showed that *snmp* of *H. armigera* was highly identical with *snmp* sequences of other insects such as *B. mori*, *H. virescens*, and *M. sexta*.

Semi-quantitative RT-PCR showed that in *H. armigera* adult, *snmp* expressed not only in female antenna and male antenna, but also in head (excluding the antennae) and legs. It was not found in thorax, abdomen or wing. Highest amounts of *snmp* were detected in antenna, and only trace amounts detected in head (excluding the antennae) and legs. No significant difference of *snmp* expression existed between female antenna and male antenna (Fig. 1: A). In order to clarify the exact expression location in the head (excluding the antenna), *snmp* was amplified in proboscis, labial palpus and maxillary palpus. The result showed that *snmp* was also expressed in these tissues (Fig. 1: B, C).

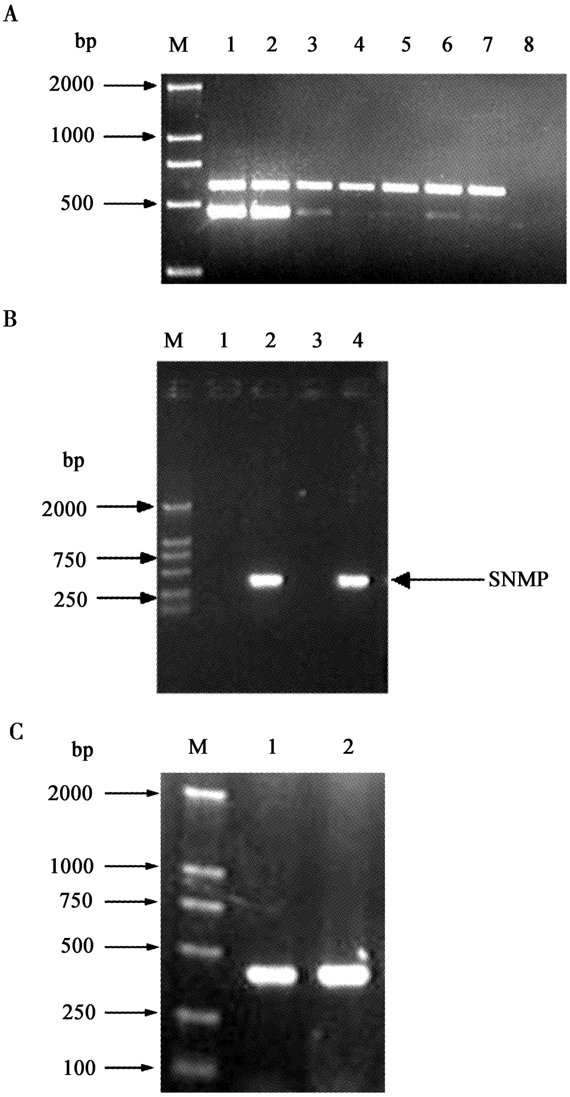

Fig. 1 Expression of *snmp* in different tissues of *Helicoverpa armigera* just after eclosion

A: Expression of *snmp* in different tissues of *H. armigera*. M: Standard molecular weight marker (DL2000); 1: Female antenna; 2: Male antenna; 3: Head (excluding the antennae); 4: Thorax; 5: Abdomen; 6: Leg; 7: Wing; 8: Without template. B: Expression of *snmp* in proboscis of *H. armigera*. M: Standard molecular weight marker (DL2000); 1: Female head (without antenna and proboscis); 2: Female proboscis; 3: Male head (without antenna and proboscis); 4: Male proboscis. C: Expression of *snmp* in labial palpus and maxillary palpus of *H. armigera*. M: Standard molecular weight marker (DL2000); 1: Female labial palpus and maxillary palpus; 2: Male labial palpus and maxillary palpus.

3.2 Construction of a DNA-BD fusion protein

Through sequencing the positive plasmid, it was confirmed that the open reading frame of *Gqα* was correct.

3.3 Transcriptional activation test and toxicity test of the DNA-BD fusion

AH109 was transformed with the hybrid construct (Gqα-pGBKT7) using a small-scale transformation protocol according to the BD Matchmaker^{TM} Library Construction and Screening Kits User Manual. The result showed that all the transformant colonies growing on SD/-Trp were white, and did not grow on SD/-Ade/-Trp, but grew on SD/-His/-Trp. There were two resolutions to eliminate the background growth of the bait strain on-His medium. One was adding 3-AT to the selection medium; the other was using SD/-Ade/-His/Leu/-Trp medium for the library screening. In this study, the latter one was adopted.

In order to test the DNA-BD fusion for toxicity, the growth rate in liquid culture of AH109 cells transformed with the "empty" DNA-BD vector was compared with that of AH109 cells transformed with the DNA-BD/bait plasmid. After 24 h shaking cultivation in the liquid culture SD/-Trp/Kan (20 μg/mL), OD_{600} of pGBKT7 was 1.339, and OD_{600} of pGBKT7-Gqα was 1.236. Both values were ≥0.8, so the DNA-BD fusion was nontoxic.

3.4 Amplification and purification of *snmp* gene

Snmp gene was successfully amplified with the primers (*snmp*-1S and *snmp*-1A) and purified with the DNA Extraction Kit.

3.5 Construction and screening of a two-hybrid library

The interaction between Gqα and SNMP was investigated by cotransformation. After 3 d-cultivation at 30℃, many clones grew on SD/-Leu/-Trp medium, but no positive clones grew on SD/-Ade/-His/-Leu/-Trp/X-α-Gal except the positive controls (Fig. 2).

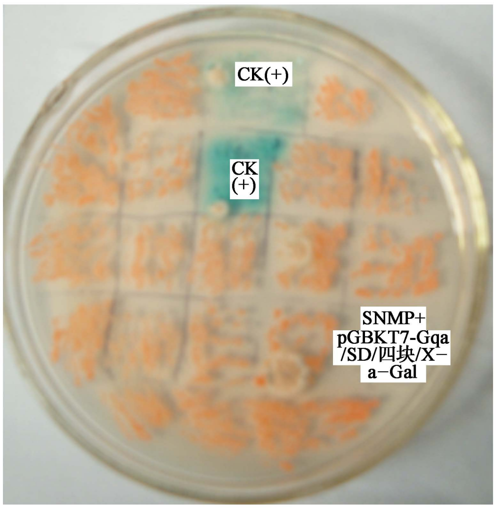

Fig. 2 Result of the interaction between Gqα and SNMP screened by nutritional markers (SD/-His/-Ade/-Leu/-Trp medium) and blue/white clone-colors (X-α-Gal)
CK(+): Positive controls; Pink clones: Negative clones.

4 DISCUSSION

In adult *H. armigera*, *snmp* was prominently expressed in the antenna, and also expressed in the head without antenna and the legs though the expression level was very low. Semi-quantitation RT-PCR results also showed that the exact expression sites in the head (excluding the antenna) were proboscis, labial palpus and maxillary palpus. Our result was inconsistent with the previously described antenna-specific expression patterns of SNMP1-*Apol* in silkmoth (*A. polyphemus*) and *snmps* in *B. mori*, *H. virescens*, and *M. sexta* (Rogers *et al.*, 1997, 2001a, 2001b). The studies mentioned above found that *snmps* specifically expressed in olfactory neurons and their developmental time of expression was in concert with the onset of olfactory function. So *snmps* were believed to be involved in the odor detection (Rogers *et al.*, 1997, 2001a, 2001b; Blomquist and Vogt, 2003). But it was found that *snmp* was also expressed in the body of *D. melanogaster* though the expression amount was much lower than that in the antenna (Benton *et al.*, 2007; Nichols and Vogt, 2007). As we know, SNMPs are homologous with CD36 protein family which has a big extracellular loop and two transmembrane domains (Rasmussen *et al.*, 1998). CD36 family members are also supposed to play roles in vertebrate taste transduction (Calder and Deckelbaum, 2006; Laugerette *et al.*, 2007). In our study, *snmp* was also expressed in proboscis, labial palpus, maxillary palpus and legs where many taste sensilla exist, which indicates that *snmp* plays some roles not only in odor detection but also in taste recognition.

It was found that GqαHarm was a ubiquitous protein in *H. armigera*, and might be involved in diverse signaling pathways (Su *et al.*, 2006). In the head, transcripts of Gqα were found in antenna, proboscis, labial palpus and maxillary palpus, however, in the head (excluding the antennae, proboscis, labial palpus or maxillary palpus), Gqα transcripts were too low to be detected by this method, which indicated that Gqα was mainly involved in both olfactory and taste function (Su *et al.*, 2006).

Does Gqα interact with SNMP? In this study, the yeast two-hybrid result indicated that Gqα did not directly interact with SNMP. Maybe SNMP forms a complex with OR and then interacts with Gqα, which needs further investigation. Hallem *et al*. (2004) found that receptor expression was necessary and sufficient for neuronal activation by food odors, that is, odorant receptors were directly activated by general food odorants. However, perception of 11-*cis*-vaccenyl acetate (cVA), a volatile pheromone in *Drosophila*, required supplemental factors not required for the detection of general food odorants. Benton *et al*. (2007) recently reported that Or67d alone failed to sensitize the empty neuron system to cVA. When SNMP was coexpressed with Or67d, high levels of cVA did elicit responses. SNMP is an inhibitory subunit in a complex with Or67d. LUSH and SNMP are members of a growing list of components required for pheromone perception but not for general odorants because pheromone signaling is more specialized compared with general odor detection. SNMP is clearly required for pheromone signaling in *Drosophila*, but the signaling mechanisms downstream of Or67d are still unknown (Jin *et al*., 2008).

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棉铃虫成虫感觉神经元膜蛋白(SNMP)表达及其与 Gqα 的关系

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摘要：昆虫嗅觉对昆虫的取食、求偶、寻找产卵场所、搜寻寄主或猎物至关重要。有研究认为感觉神经元膜蛋白(SNMP)和 Gq 蛋白 α 亚基(Gqα)参与信号转导。为了阐明 *snmp* 的空间表达情况并确定 Gqα 是否与 SNMP 直接结合，我们采用半定量 RT-PCR 技术对 *snmp* 在棉铃虫 *Helicoverpa armigera* (Hübner)成虫的触角、头、胸、腹、足、翅、喙、下颚须和下唇须的分布情况进行了研究，并利用酵母双杂交技术对 SNMP 与 Gqα 的关系进行了研究。半定量 RT-PCR 研究发现，*snmp* 不仅在棉铃虫成虫的触角中表达，而且在喙、下颚须、下唇须及足上都有表达。利用酵母双杂交技术研究发现，Gqα 与 SNMP 不直接相互作用，Gqα 不是 SNMP 的直接下游结合蛋白。这些研究结果说明,SNMP 不仅参与气味识别而且也参与味觉识别；SNMP 可能与气味受体(OR)形成复合物，然后与 Gqα 结合，这需要我们进一步深入的研究进行证明。

关键词：棉铃虫；昆虫嗅觉；信号转导；SNMP；Gqα；酵母双杂交技术；空间表达

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